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A Simple Multichannel Fluidic System for Laminar Flow Over Planar Substrates

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14. ABSTRACT

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A Simple Multichannel Fluidic System for Laminar Flow over Planar Substrates

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EXECUTIVE SUMMARY

We describe the design, assembly, and operation of a multichannel fluidic system for performing laminar flow biochemical assays on planar substrates under optical inspection using standard laboratory microscopes. The basic design uses a reusable elastomer gasket, which forms the fluidic channel, sandwiched between the substrate and a sapphire plate having integrated microfluidic connections. Designs for use with both upright and inverted microscopes are described. The devices are easy to construct and can be quickly assembled, with all but one component easily fabricated in one day. The system allows multiple assays to be performed on a single substrate, including a standard microscope slide, allowing substrate surface chemistry, assay chemistry, and fluidic protocols to be rapidly evaluated for assay labels that can be optically detected. Different molds for the PDMS gasket can be quickly machined, allowing for rapid design and testing of different channel geometries. Although this system was specifically designed for developing fluidic force discrimination assays using microbead labels for use with NRL BARC™ biosensor chips, it could be readily adapted for other lab-on-a-chip applications and other detection schemes.

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INTRODUCTION

A common approach to detecting biological molecules is to capture them on a solid substrate that has been functionalized with receptor molecules (e.g. antibodies) specific to that target, and then attach to the captured target molecules labels that produce some type of detectable signal. Traditionally, both the capture and labeling in such an assay is accomplished using biomolecular recognition between the target molecule and specific receptors, with the label most often including a radioisotope, enzyme, or fluorescent molecule. Detection methods have been developed based on a wide range of transduction mechanisms, including optical, electrical, electrochemical, thermal, and piezoelectrical means, which are covered eloquently by numerous reviews (see, for example [1-2]). Recently, magnetic particles have been developed as labels for biosensing [3]. Magnetic particles have several potential advantages over other labels. They are not subject to degradation over time or photobleaching, making their properties very stable. When detected with magnetic sensors, there is typically no similar background signal in biological samples, and the signal is immune to any chemical or optical interference.

A key component of the assay (the capture and labeling) is the method by which sample containing the target, along with any other required reagents, are delivered to the capture surface. Most often they are delivered in a static fluidic environment, such as a microtiter well. More recently, a variety of microsystems have been developed to deliver the fluids under dynamic (often laminar) flow over planar substrates [see e.g. 4-10]. Such microfluidic systems are central to the development of "lab-on-a-chip" applications that offer a variety of potential sensing advantages, including smaller sample volumes, reduced reagent consumption, more easily controlled process parameters, and—most importantly—faster results without compromising sensitivity or selectivity.

BACKGROUND

The Naval Research Laboratory (NRL) is developing the compact Bead Array Sensor System (*cBASS*™) for multiplexed detection of proteins, bacteria, and viruses, including nucleic acids and toxins. This system uses magnetic microbeads to label biomolecules captured onto a receptor-patterned microchip that contains an embedded array of magnetic microsensors [11-16]. The sensors in the Bead ARray Counter (BARC™) microchip are micron-scale wire-like structures made with giant magnetoresistive (GMR) material. When a magnetic bead is present above a GMR sensor, the resistance decreases by a detectable amount; the more beads present, the larger the decrease. Hence, the BARC™ approach replaces the optical system required for fluorescence-based detection, for example, with simple, sensitive, and compact microelectronics.

The assay on the BARC™ chip is performed in a microliter volume fluid cell under laminar flow conditions. In addition to improving the capture and labeling of any targets in the sample, [17], the laminar flow can be adjusted to apply controlled fluidic forces to the microbeads on the chip surface in order to selectively remove those that are not specifically labeling captured target molecules [18]. This unique assay step, called fluidic force discrimination (FFD), greatly reduces unwanted background signal, enabling the rapid identification of captured biomolecules with high sensitivity and specificity with little or no sample processing. Highly sensitive multiplexed DNA assays (<10 fM) and immunoassays (<50 pg/mL) have been demonstrated in less than 20 minutes, without amplification or preconcentration steps, using a variety of complex sample matrices such as blood and food products [19].

Although the use of magnetic labels and chip-based magnetoelectronic detection provides many key advantages of the NRL *cBASS*™, including scalable, multiplexed detection and compact instrumentation, the performance of the system depends principally on the assay (which

ultimately determines how many beads are available for detection). The performance of the assay depends on the chip surface chemistry, which must promote the target-receptor interactions while concurrently inhibiting non-specific interactions, and the protocols by which the assay is performed, including the flow rates for sample and reagent delivery and FFD. Therefore, the assay performance is independent of the magnetoelectronics which counts the beads, and can be optimized separately from the magnetoelectronics.

Since BARC chips are a valuable resource, it is highly desirable to optimize assay protocols without using a chip for every experiment. The microbead labels are readily observed under a conventional optical microscope, so beads captured in an assay can be counted optically. Therefore, if assays can be performed on a substrate surface with identical chemistry to that used on a chip, and with fluidic conditions that mimic those used on a chip, then assays can be optimized without consuming BARC chips. It is also desirable to conduct multiple such assays in parallel on a single substrate to help mitigate substrate-to-substrate variations during assay development.

Examples of fluidic devices designed to handle multiple samples or assay protocols include inventions by Rosenberg [20], Elkins [21], Bolz and DeForest [22], Golias [23], Clatch [24], Wilding *et al*. [25], and Leatzow *et al*. [26]. There are also a number of commercially available slides incorporating multiple fluidic compartments or the means to create individual chambers on the slide (e.g., Fisher Scientific, Grace Bio-Labs). We have previously designed and manufactured various custom microliter volume flow cells made of quartz or molded from polydimethylsiloxane (PDMS), as well as a multi-well, flow-through hybridization chamber which incubate three whole chips in parallel for magnetic force discrimination assays [12]. The approaches taken by these devices are guided by the applications addressed. For example,

devices may isolate separate volumes on a single microscope slide in order to analyze several samples at once (in static volumes). Other devices contain a single channel for the purpose of analyzing individual particles. In general, however, none of these—with the exception of the inventions by Wilding *et al.*, Leatzow *et al.*, and those from our laboratory—are appropriate for conducting assays under controlled flow rates.

Although the devices by Clatch and Wilding *et al.* can both be used for monitoring different reactions or assay conditions in parallel, the devices as reported require complex semiconductor microfabrication methods, are designed to share reagents from a single reservoir, or the reagents are distributed by uncontrolled capillary action. The fluidic device and its variants by Leatzow *et al*. have some similarities to the device we report here. However, significant differences lie in the channel dimensions and compression method used. Their device relies on predefined channel geometries of millimeter dimensions milled into an acrylic manifold. This design makes it inconvenient to change the channel geometry, and the relatively large channel dimensions require greater fluid volumes. Here we describe a more flexible, reusable, multichannel microfluidic system designed for conducting heterogeneous assays on a solid substrate using laminar flow and optical inspection.

TECHNICAL APPROACH

We have designed versions of our multichannel fluidic system to accommodate both upright (Figure 1) and inverted microscopes **(**Figure 2). The system is comprised of the following four basic components (Figure 3): 1) a sapphire top plate with attachments for fluidic I/O connections; 2) a PDMS gasket, cast from a precision-milled mold, that forms a flow channel between the sapphire and substrate; 3) a flat substrate, such as a microscope slide or silicon wafer section, on which the assay binding occurs; and 4) an aluminum microscope stage dock

designed to easily compress the components together and form a water tight seal, and to allow all the channels to be optically inspected with standard microscope objectives.

Sapphire top plate. Sapphire was chosen as the top plate material for its rigidity under a combination of point and distributed compressive loads along with its optical clarity. Although its overall design is simple, it is the most expensive and specialized component to manufacture. The use of sapphire became necessary to address a problem of early models of the system that used a polymethylmethacrylate (PMMA) top plate. The PMMA plate bowed under compression by the retaining ring, causing variable pressure across the PDMS gasket that allowed leaking between channels. The additional stiffness of the sapphire top plate provides uniform pressure over the gasket preventing such leakage. The ports for connecting tubing and directing flow to the PDMS-defined channels were blind milled along the edge of the plate by conventional diamond tip tooling (Insaco Inc., Quaktertown, PA). The sapphire top plate is connected to Tygon™ microbore tubing (Saint-Gobain Performance Plastics, Akron, OH) via 26 gauge hypodermic stainless-steel 316 tubing secured into the side ports with Hardman Double-Bubble® epoxy or silicone adhesive (Royal Adhesives and Sealants LLC, South Bend, IN).

PDMS gasket. The PDMS gasket defines the boundaries of the channels—height, width and length. PDMS (SYLGARD® 184, Dow Corning, Midland, MI) has received much attention over the years for its many desirable features, including low cost, transparency, ease of fabrication, biocompatibility, and ability to form an irreversible seal to flat, glassy substrates (under appropriate conditions) [27]. In its pre-polymerized, liquid state, PDMS easily conforms to molds of varying designs and then quickly polymerizes when heated, making rapid prototyping of different molds and channel designs feasible.

As an example, gaskets for the upright microscope version are created in the aluminum mold shown in Figure 4A. Each gasket creates five lanes that are 800 μm wide and 250 μm tall (Figure 5A). These dimensions were determined to effectively emulate the laminar conditions desired over the center of a BARC III chip [16]. The mold was designed in Autodesk Inventor®, (Autodesk, Inc., San Rafael, CA) and produced using a CNC milling machine (Haas Automation, Inc., Oxnard, CA). To fabricate a gasket, a 9:1 ratio of pre-polymer to curing agent is hand mixed in a glass vial and then placed in a vacuum desiccator to degas the mixture. The liquid PDMS mixture is then poured into the aluminum mold, sealed, and placed in an oven for 30 min at 70 °C. Finally, the resulting PDMS gasket is carefully peeled away from the mold and positioned over the substrate.

The mold shown in Figure 4B was designed for use in an inverted microscope, which requires optically clear substrates. It creates a PDMS gasket that forms three walls of the flow channel (top and sides), with the assay substrate being the bottom, or fourth wall (Figure 5B). The optically clear PDMS gasket allows condenser light through the top of the channel to illuminate the substrate, and also permits the easy fabrication of different channel depths (down to 25 μm) and geometries [see for example, Ref. 28].

PDMS gaskets with an overall thickness less than 250 μm have been found difficult to place on substrates without tearing, making this the minimum channel height for the version used with the upright microscope. For the inverted microscopy system, the gasket is nominally 500 μm thick, enabling the formation of much shallower channels; heights as low as 25 μm have been made, only limited by CNC machining tolerances.

Assay substrate. The assay is performed on a planar substrate functionalized with biomolecular capture probes and a non-fouling coating. The substrate must be flat enough to

form a good seal with the PDMS gasket. Commonly used substrates in our lab include standard 75 mm × 25 mm glass microscope slides and silicon wafers diced to similar dimensions. For multiplexed assays within a single channel, multiple capture probes (e.g. antibodies or oligonucleotides) can be spotted down the channel either manually or via a robotic arrayer [29]. It should be noted that the geometry is designed so that the substrate is the bottom of the channel, so that the microparticle labels, which have specific gravity greater than the solvent (aqueous buffers), will settle on the substrate surface via gravity.

Microscope stage dock. There are two configurations of the fluidic system, an upright microscope version that can be used in reflectance, and an inverted microscope version that can be used in reflectance or transmission modes. The upright system is used when the substrate is opaque. The sapphire forms the top of the fluidic channel, allowing the user to clearly image the substrate surface using the objectives above the microscope stage (Figure 6). With optically clear substrates, the inverted microscope can be used with the alternate dock that uses the substrate and sapphire as the bottom and top of the channel, respectively (Figure 7). The assay surface (the top surface of the substrate), is then viewed with the objectives below the microscope stage. The PDMS gasket is sandwiched and compressed between the sapphire plate and the substrate by a retaining ring, forming a water tight seal. The compression force is controlled by four screws that secure the retaining ring to the aluminum microscope stage dock. The retaining ring, microscope dock, and sapphire top window are reusable. In both versions of the device the assay substrate is replaced or regenerated between experiments, but the PDMS gasket can be reused until it no longer provides a reliable, water-tight seal due to aging (typically 1-2 weeks).

OPERATION

A typical application of the multi-channel platform would be as follows [19]. A metal coated (e.g. Au, Ag, Al, or Al_xO_y) glass slide is functionalized with an anti-fouling coating and chemistry designed for immobilizing biomolecular capture probes. The PDMS gasket is carefully aligned over the substrate and the exposed surface within the lanes is then arrayed with probes such as antibodies or single-stranded DNA (ssDNA) **(**Figure 8). The individual components of the multi-channel platform are assembled together, mounted on a microscope stage, and connected to a peristaltic pump. The majority of experiments performed in our laboratory have the fluidics under negative pressure. Therefore the pump is attached to the outlet tubing from a channel and the fluids are pulled through the system. The inlet tubing to each channel is submerged in a container containing the assay reagents (Figure 9). A typical result of these individual FFD assays with optical counting of the microbead labels is shown in Figure 10. The dose-response results are for 2-probe assays performed for a specific gene sequence from *Bacillus anthracis* (BA) using 21 base ssDNA capture probes, 54 base synthetic ssDNA sample in buffer, and 18 base ssDNA label probes. In our multichannel fluidic system we are able to achieve fM detection limits in <30 minutes at room temperature without sample amplification.

CONCLUSIONS

We have designed a convenient multichannel fluidic system to easily perform laminar flow biochemical assays on planar substrates under optical inspection using standard laboratory microscopes. The device is easy to construct and can be quickly assembled—everything, with the exception of the sapphire top plate, can be fabricated in one day. Different molds for the PDMS gasket can also be quickly machined, allowing for rapid design and testing of different channel geometries. For the design compatible with upright microscopy, channel heights of 250

μm can be easily achieved; in the inverted microscopy design, channel heights as low as 25 μm have been made. The flexibility of this device creates the ability to study other channel sizes and shapes, such as channels to create compressive flow between two laminar streams or intersecting channels for studies of fluid mixing.

Although this system was specifically designed to rapidly develop fluidic force discrimination assays on inexpensive planar substrates using optically-detected microbead labels, it could be readily adapted for other lab-on-a-chip applications and other detection schemes, especially those where optical microscopy can be used to characterize the outcome of the assay (e.g. via fluorescence or chemiluminescence). The design could also be adapted for multiplexed fluidic addressing of arrays of solid state detectors mounted within or under planar substrates, allowing reversible mounting of the detector to the fluidics with reusable fluidic components.

ACKNOWLEDGEMENTS

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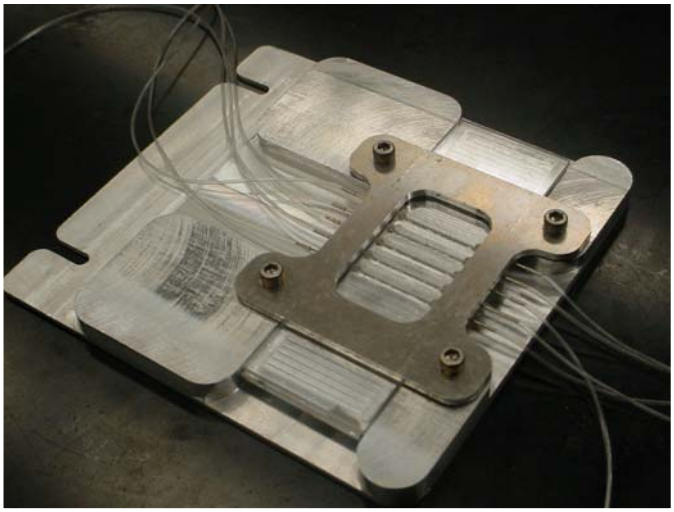

Figure 1. A fully assembled multichannel fluidic system. This version mounts onto the stage of a standard upright microscope.

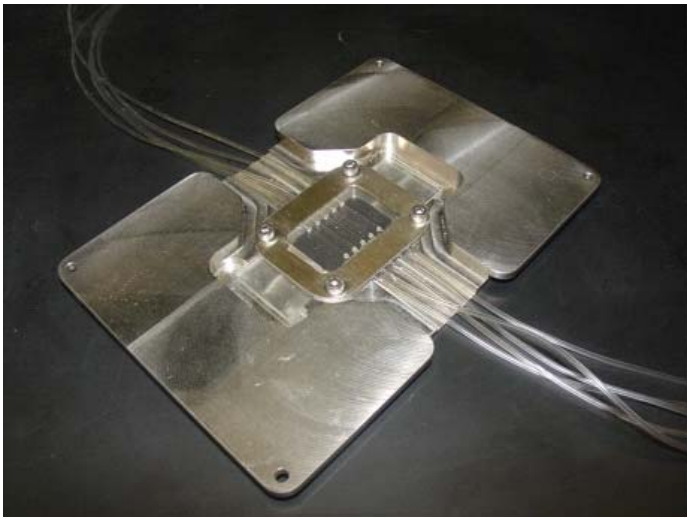

Figure 2. A fully assembled multichannel fluidic system for use with an inverted microscope. Note that a transparent substrate is required.

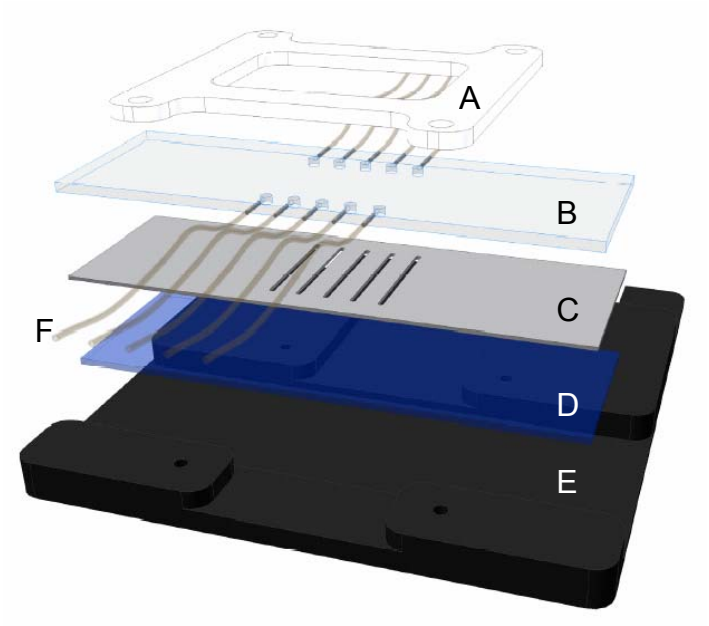

Figure 3. Exploded view of the standard microscope multichannel fluidic system assembly: (A) retaining ring; (B) sapphire top plate; (C) PDMS gasket with defined channels; (D) assay substrate; (E) aluminum microscope stage dock; and (F) Tygon™ microbore tubing.

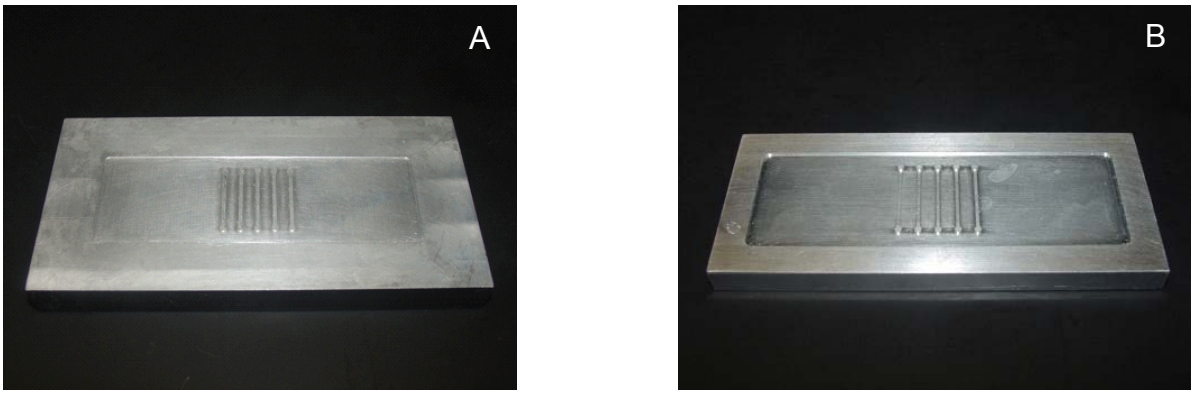

Figure 4. Aluminum PDMS molds containing (A) five lanes that are 800 µm wide, 250 µm high, and 15 mm long; and (B) 800 µm wide, 15 mm long, and varying in height from 25 µm (far left) to 250 µm (far right).

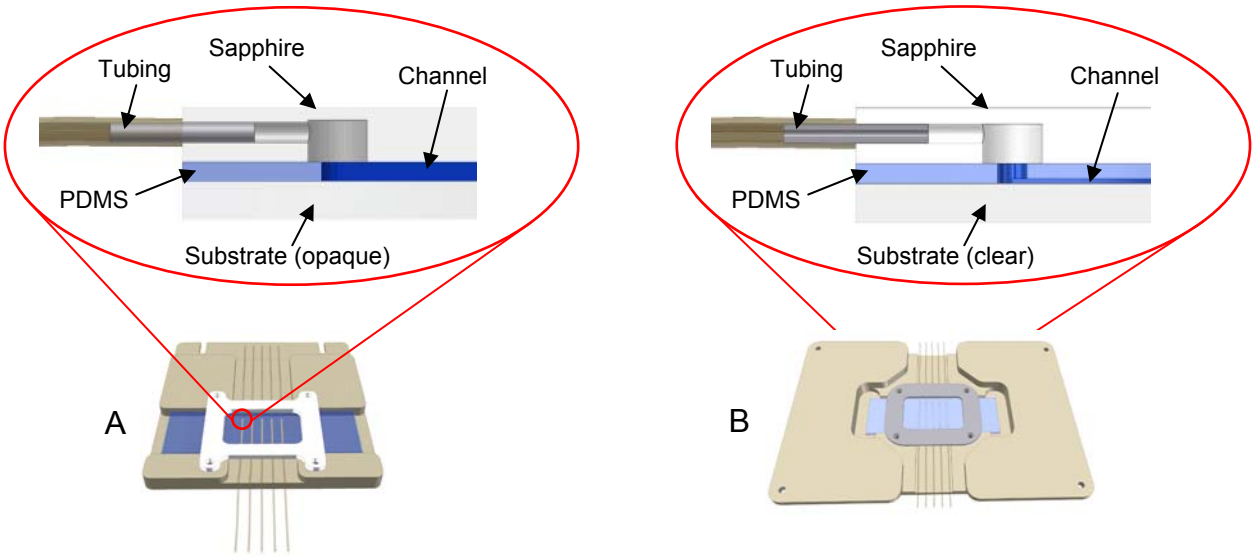

Figure 5. Cross-sectional view of the fluidic interconnects for (A) the standard microscope, and (B) the inverted microscope multichannel fluidic system. Note that in (A) the sapphire top plate acts as the top face of the microchannel and is therefore in direct contact with assay fluids that flow down the lane. This geometry is necessary so that unobstructed observation of the opaque substrate surface can be made with a standard microscope. In (B) the microchannel height can be defined by varying the depth of the channel embossed into the PDMS gasket. With the inverted microscope, the surface of the clear substrate is observed through the substrate from below.

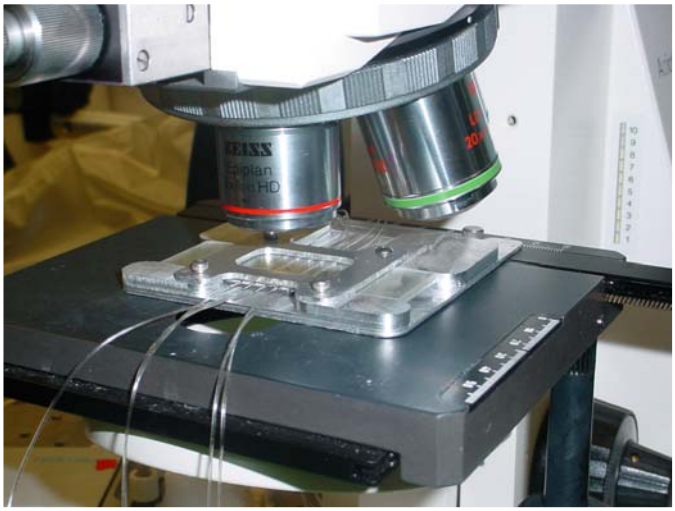

Figure 6. The standard microscope multichannel fluidic system in use.

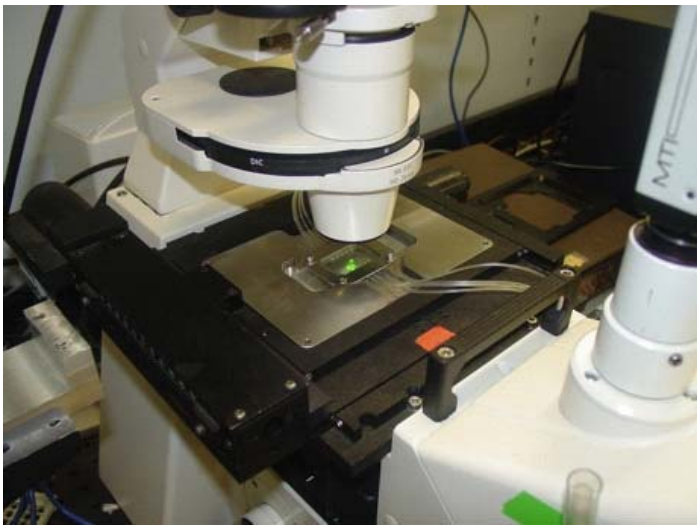

Figure 7. The inverted microscope multichannel fluidic system in use.

Figure 8. Close-up view of three 800 µm-wide lanes, where two have a series of DNA probe spots arrayed on the exposed substrate.

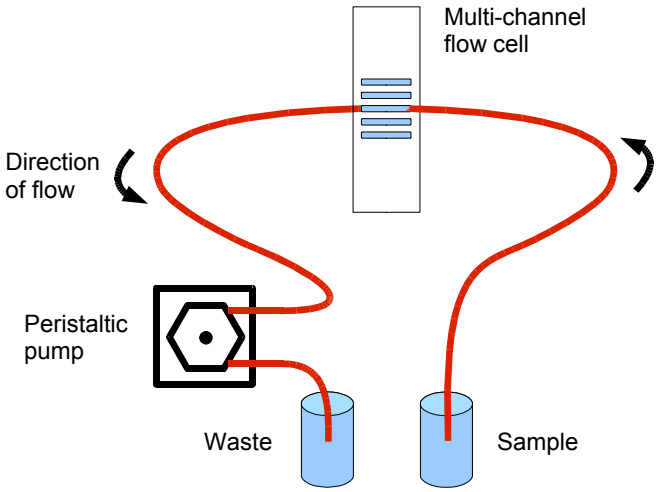

Figure 9. Typical experimental set-up for fluid flow under negative-pressure.

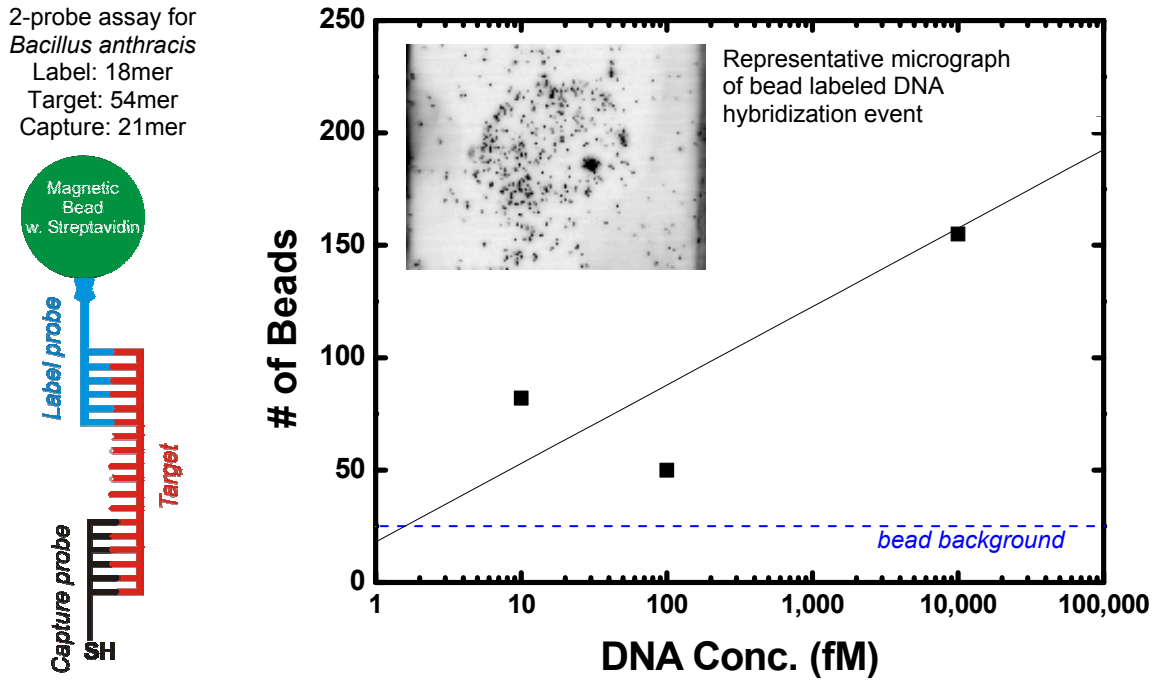

Figure 10. Results of an example two-probe DNA assay for *Bacillus anthracis* (BA) performed with the multichannel fluidic system. This experiment optically determined the microbead number within a capture spot as a function of DNA concentration. Results were fM limits of detection in 30 min. at room temperature without the use of PCR. The bead background is ~25 beads per spot.